IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

APPEAL BRIEF FOR THE APPELLANTS

Ex parte KILGER et al.

METHOD FOR THE UNCOUPLED, DIRECT, EXPONENTIAL AMPLIFICATION AND SEQUENCING OF DNA MOLECULES WITH THE ADDITION OF A SECOND THERMOSTABLE DNA POLYMERASE AND ITS APPLICATION

Serial Number: 09/339,103
Filed: June 24, 1999
Appeal No.:
Group Art Unit: 1637
Examiner: Kenneth R. Horlick

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Respectfully submitted,

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In re the application of: Confirmation No: 2157

KILGER et al. Art Unit: 1637

Application No.: 09/339,103 Examiner: Kenneth R. Horlick

Filed: June 24, 1999 Atty Docket No. 101614-08090

For: METHOD FOR THE UNCOUPLED, DIRECT, EXPONENTIAL AMPLIFICATION AND SEQUENCING OF DNA MOLECULES WITH THE ADDITION OF A SECOND THERMOSTABLE DNA POLYMERASE AND ITS APPLICATION

BRIEF ON APPEAL

Date: April 26, 2004

I. INTRODUCTION

This is an appeal from the action of the Examiner dated May 30, 2003, finally rejecting claims 1-126, 132-137 and 141-146, all of the claims pending in this application, as being unpatentable over certain prior art under 35 U.S.C. 103 and under the judicially created doctrine of obviousness-type double patenting. A Notice of Appeal was timely filed on December 1, 2004 with a Petition for Extension of Time. This Brief is being timely filed with a Petition for Extension of Time.

II. REAL PARTY IN INTEREST

The real party in interest in present application on appeal is Roche Diagnostics GmbH.

III. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences known to the Appellant, Appellants' representative or the assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

IV. STATUS OF CLAIMS

Claims 1-126, 132-137 and 141-146, all of the claims pending in the present application are being appealed.

V. STATUS OF AMENDMENTS

A Request for Reconsideration was timely filed on October 21, 2003. The Request for Reconsideration did not include any amendments therein.

VI. SUMMARY OF THE INVENTION

Some embodiments of the present invention relate to a method and/or a kit for sequencing at least a portion of a RNA involving converting the RNA to a DNA and simultaneously amplifying the DNA and generating full length and truncated copies of the DNA for sequencing, comprising the steps of (a) subjecting a mixture in a single step to a thermocycling reaction, the thermocycling reaction comprises heat denaturation, annealing and synthesis, wherein said mixture comprises said RNA, a buffer solution, a first primer which is able to hybridize with a strand of said DNA, a second primer which is able to hybridize with a strand of said DNA complementary to the strand with which the first primer is able to hybridize, wherein at least one of the first and second primers is labeled, deoxynucleotides or deoxynucleotide derivatives,

wherein said deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP, at least one dideoxynucleotide or another terminating nucleotide, and at least two thermostable DNA polymerases, wherein said at least two thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide compared with said first thermostable DNA polymerase, wherein one of said at least two thermostable DNA polymerases has reverse transcriptase activity, to generate full-length and truncated copies of said DNA, wherein the full-length copies have a length equal to that of at least a portion of said DNA spanning the binding sites of the first and second primers; (b) separating at least said truncated copies to make a sequence ladder; and thereafter (c) reading the sequence ladder to obtain the sequence of said at least a portion of said RNA wherein the conversion of the RNA to the DNA is conducted in the presence of the at least two thermostable polymerases.

Other embodiments of the present invention are directed to a method for sequencing at least a portion of a DNA involving simultaneously amplifying the DNA and generating full length and truncated copies of the DNA for sequencing, comprising the steps of (a) subjecting a mixture in a single step to a thermocycling reaction, the thermocycling reaction comprises heat denaturation, annealing and synthesis, wherein said mixture comprises said DNA, a buffer solution, a first primer which is able to hybridize with a strand of said DNA, a second primer which is able to hybridize with a strand of said DNA complementary to the strand with which the first primer is able to

hybridize, wherein at least one of the first and second primers is labelled, deoxynucleotides or deoxynucleotide derivatives, wherein said deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP, at least one dideoxynucleotide or another terminating nucleotide, at least two thermostable DNA polymerases, wherein said at least two thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide compared with said first thermostable DNA polymerase, and at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule, wherein said at least one polymerase-inhibiting agent is a compound having at least one acid anhydride group per molecule, to generate full-length and truncated copies of said DNA, wherein the full-length copies have a length equal to that of at least a portion of said DNA spanning the binding sites of the first and second primers; (b) separating at least said truncated copies to make a sequence ladder; and thereafter (c) reading the sequence ladder to obtain the sequence of said at least a portion of said DNA. The at least one polymerase-inhibiting agent that loses inhibitory ability is enables a "hot-start" of the generation of full-length and truncated copies of the DNA.

VII. THE FINAL REJECTIONS

Claims 1-126. 132-137 and 141-146 are pending in this application. No claim stands allowed.

Claims 1-126, 134-137 and 143-146 were finally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 34-58 of copending Application No. 09/257,166 in view of Gelfand et al. (U.S. Patent Application No. 5,310,652) and Birch et al. (U.S. Patent Application No. 5,677,152).

Claims 1-126, 134-137 and 143-145 were finally rejected under 35 U.S.C. 103(a) as being obvious over the combination of Köster et al. (U.S. Patent No. 5,928,906) in view of Gelfand et al. and Birch et al.

Claims 132-133 and 141-142 were finally rejected under 35 U.S.C. 103(a) as being obvious over the combination of Köster et al. in view of Gelfand et al. and Birch et al. and further in view of Hill (U.S. Patent No. 5,525,492).

VIII. ISSUES ON APPEAL

The first issue on appeal is whether claims 1-126, 134-137 and 143-146 would have been obvious over the combination of copending Application No. 09/257,166 in view of Gelfand et al. (U.S. Patent Application No. 5,310,652) and Birch et al. (U.S. Patent Application No. 5,677,152).

The second issue on appeal is whether claims 1-126, 134-137 and 143-145 would have been obvious over the combination of Köster et al. (U.S. Patent No. 5,928,906) in view of Gelfand et al. and Birch et al.

The third issue on appeal is whether claims 132-133 and 141-142 would have been obvious over the combination of Köster et al. in view of Gelfand et al. and Birch et al. and further in view of Hill (U.S. Patent No. 5,525,492).

IX. GROUPING OF CLAIMS

Each claim of this patent application is separately patentable, and upon issuance of a patent will be entitled to a separate presumption of validity under 35 U.S.C. 282. For convenience in handling of this appeal, claims 1-63 stand together. Claims 64-126, 132-137 and 141-146 separately stand together, in that they require a polymerase-inhibiting agent.

X. APPELLANT'S ARGUMENTS

Legal Overview

Several basic factual inquiries must be made to determine obviousness or non-obviousness of patent application claims under 35 U.S.C. § 103. These factual inquiries are set forth in Graham v. John Deere Co., 383 U.S. 1, 17, 148 U.S.P.Q. 459, 467 (1996):

> Under § 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; the level of ordinary skill in the pertinent art resolved. Against this backdrop, the obviousness or non-obviousness of the subject matter is determined.

The specific factual inquiries set forth in *Graham* have not been considered or properly applied by the Examiner formulating the rejections of the claims. Particularly the differences between the prior art and the claims were not properly determined. As stated by the Federal Circuit in In re Ochiai, 37 U.S.P.Q. 2d 1127, 1131 (Fed. Cir. 1995):

> [t]he test of obviousness *vel non* is statutory. It requires that one compare the claim's subject matter

> as a whole with a prior art to which the subject matter pertains. 35 U.S.C. § 103.
>
> The inquiry is highly fact-specific by design.... When the references cited by the Examiner fail to establish a *prima facie* case of obviousness, the rejection is improper and will be overturned. In re Fine, 837 F.2d 1071, 1074, 5 U.S.P.Q. 2d 1596, 1598 (Fed. Cir. 1988). (Emphasis added.)

When rejecting claims under 35 U.S.C. § 103, an Examiner bears an initial burden of presenting a prima facie case of obviousness. A prima facie case of obviousness is established only if the teachings of the prior art would have suggested the claimed subject matter to a person of ordinary skill in the art. If an Examiner fails to establish a prima facie case, the rejection is improper and will be overturned. See: In re Rijckaert, 9 F.3d 1531, 28 U.S.P.Q. 2d. 1955 (Fed. Cir. 1993). "If examination.... does not produce a prima facie case of unpatentability, then without more the applicant is entitled to the grant of the patent." In re Oetiker, 977 F.2d 1443, 1445 - 1446 24 U.S.P.Q. 2d. 1443, 1444 (Fed. Cir. 1992).

Appellants respectfully submit that the Examiner has not made a proper *prima facie* rejection under 35 U.S.C. § 103(a), because the combination of prior art references cited fails to teach or suggest the present invention and because it would not have been obvious to combine the cited references.

The presently claimed invention is directed to a method and/or a kit for sequencing at least a portion of a RNA involving converting the RNA to a DNA and simultaneously amplifying the DNA and generating full length and truncated copies of the DNA for sequencing, comprising the steps of (a) subjecting a mixture in a single step to a thermocycling reaction, the thermocycling reaction comprises heat

denaturation, annealing and synthesis, wherein said mixture comprises said RNA, a buffer solution, a first primer which is able to hybridize with a strand of said DNA, a second primer which is able to hybridize with a strand of said DNA complementary to the strand with which the first primer is able to hybridize, wherein at least one of the first and second primers is labeled, deoxynucleotides or deoxynucleotide derivatives, wherein said deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP, at least one dideoxynucleotide or another terminating nucleotide, and at least two thermostable DNA polymerases, wherein said at least two thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide compared with said first thermostable DNA polymerase, wherein one of said at least two thermostable DNA polymerases has reverse transcriptase activity, to generate full-length and truncated copies of said DNA, wherein the full-length copies have a length equal to that of at least a portion of said DNA spanning the binding sites of the first and second primers; (b) separating at least said truncated copies to make a sequence ladder; and thereafter (c) reading the sequence ladder to obtain the sequence of said at least a portion of said RNA wherein the conversion of the RNA to the DNA is conducted in the presence of the at least two thermostable polymerases.

Other embodiments of the present invention are directed to a method for sequencing at least a portion of a DNA involving simultaneously amplifying the DNA and generating full length and truncated copies of the DNA for sequencing, comprising the

steps of (a) subjecting a mixture in a single step to a thermocycling reaction, the thermocycling reaction comprises heat denaturation, annealing and synthesis, wherein said mixture comprises said DNA, a buffer solution, a first primer which is able to hybridize with a strand of said DNA, a second primer which is able to hybridize with a strand of said DNA complementary to the strand with which the first primer is able to hybridize, wherein at least one of the first and second primers is labelled, deoxynucleotides or deoxynucleotide derivatives, wherein said deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP, at least one dideoxynucleotide or another terminating nucleotide, at least two thermostable DNA polymerases, wherein said at least two thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide compared with said first thermostable DNA polymerase, and at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule, wherein said at least one polymerase-inhibiting agent is a compound having at least one acid anhydride group per molecule, to generate full-length and truncated copies of said DNA, wherein the full-length copies have a length equal to that of at least a portion of said DNA spanning the binding sites of the first and second primers; (b)

separating at least said truncated copies to make a sequence ladder; and thereafter (c) reading the sequence ladder to obtain the sequence of said at least a portion of said DNA. The at least one polymerase-inhibiting agent that loses inhibitory ability is enables a "hot-start" of the generation of full-length and truncated copies of the DNA.

The Examiner has noted that Köster et al. disclose methods "requiring two different polymerases..." The Examiner has also noted that the Köster et al. patent does not disclose DNA polymerase-mediated reverse transcription coupled to PCR amplification. However, the Examiner has asserted that one of ordinary skill in the art would have been motivated to modify the method of Köster et al. by application towards RNA using a polymerase with reverse transcriptase activity because Gelfand et al. disclosed the advantages of combined reverse-transcription and amplification.

However, Appellants respectfully submit that Köster et al. already discloses that the nucleic acid molecule in their process can be "RNA if combined with reverse transcription to generate a DNA. For example, reverse transcription can be performed using a suitable reverse transcriptase (e.g., Moloney murine leukemia virus reverse transcriptase) using standard techniques..." (col. 6, lines 23-35).

None of the cited references teach or suggest using a first DNA polymerase to amplify DNA to produce truncated copies of a DNA and a second DNA polymerase to generate full length copies of the DNA, as required by the present claims.

There would have been no reason to modify Köster et al. towards RNA since Köster et al. already contemplates a combined transcribing, amplifying and sequencing method in which a reverse transcriptase (e.g., Moloney murine leukemia virus reverse transcriptase) is used to generate a DNA and then two different polymerase enzymes,

each having a different affinity for the particular chain terminating nucleotide, are used.

Appellants respectfully note that Köster et al. nowhere teaches or suggests that the reverse transcriptase (e.g., Moloney murine leukemia virus reverse transcriptase) also be used as one of two polymerase enzymes for sequencing.

Gelfand et al. fails to make up for deficiencies in Köster et al. since Gelfand et al. also fails to suggest a reverse transcriptase used for transcribing (e.g., Moloney leukemia virus reverse transcriptase) also be used as one of two polymerase enzymes for sequencing.

Additionally, there would have been no motivation to replace one of the two Köster et al. polymerase enzymes with a polymerase of Gelfand et al. since any Köster et al. RNA would have already been converted to CDNA using the "suitable reverse transcriptase (e.g., Moloney murine leukemia virus reverse transcriptase) using standard techniques."

Furthermore, Appellants respectfully note that none of the applied references teach or suggest conducting transcription in the presence of at least two thermostable DNA polymerases. The present claims require that "the conversion of the RNA to the DNA is conducted in the presence of the at least two thermostable DNA polymerases." Applicants do not believe that any proper motivation has been or could be shown to modify any of the teachings of the cited references to reverse transcribe in the presence of two thermostable DNA polymerases.

Additionally, regarding claims requiring "at least one polymerase-inhibiting agent," Applicants respectfully note that none of the applied references teach or suggest

such a "hot-start" agent in a sequencing reaction with at least the thermostable polymerases, as required in the present claims 64-99 and 122-144.

The Examiner has agreed that no reference teaches or suggests conducting reverse transcription in the presence of two thermostable DNA polymerases. However, the Examiner has asserted that while "no single reference teaches or suggests carrying out this two polymerase reverse transcription, it is nevertheless submitted that the combination of the references does suggest it." (page 7, lines 12-15 of the Office Action, emphasis in original).

However, Appellants submit that the Examiner has not established a prima facie case of obviousness. In particular, as noted in Section 2143.03 of the U.S. Patent and Trademark Manual of Patent Examining Procedure, "[t]o establish prima facie obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art".

The present claims include a limitation that conversion of RNA to DNA "is conducted in the presence of ...at least two thermostable DNA polymerases" (see present claim 1).

Applicants respectfully note that Gelfand et al. does not teach or suggest conversion of RNA to DNA in the presence of at least two thermostable DNA polymerases. In particular, Gelfand et al. actually teaches against the inclusion of two polymerases by stating that the Gelfand et al. process requires "only one enzyme where previous methods required two" (Gelfand et al. column 6, lines 34-36).

Appellants additionally note that Köster et al. does not teach or suggest conversion of RNA to DNA in the presence of at least two thermostable DNA

polymerases. In particular, Köster et al. discloses that "reverse transcription can be performed using a suitable reverse transcriptase (e.g. Moloney murine leukemia virus reverse transcriptase)" (Köster et al. column 6, lines 27-31). Thus, Köster et al. clearly disclose reverse transcription using only a single reverse transcriptase.

As neither Gelfand et al. nor Köster et al. teach or suggest conversion of RNA to DNA in the presence of at least two thermostable DNA polymerases, Appellants respectfully submit that the combination of Gelfand et al. and Köster et al. does not and can not teach or suggest conversion of RNA to DNA in the presence of at least two thermostable DNA polymerases.

The Examiner has further asserted that considering the Gelfand et al. and Köster et al. "references together would have been suggestive of obtaining the combined advantages of both references-using RNA and the polymerase of Gelfand et al. as one of the two polymerases in the method of Köster et al."

However, Appellants respectfully note that Köster et al. already disclose using RNA. Appellants additionally note that Köster et al. already teach using the same polymerase (for examples including Taq and Tth, see column 7, lines 53-62 of Köster et al.) of Gelfand et al. (for examples including Taq and Tth, see column 13, line 68, to column 14, line 9).

Thus, Köster et al. already discloses using RNA and also already discloses the polymerase of Gelfand et al. as one or the polymerases in their method. Thus, even if the particular polymerase of Gelfand et al. was used in Köster et al., which Köster et al. already discloses, such a combination would not achieve the advantages achieved by the presently claimed invention.

Thus, the presently claimed invention would not have been recreated even with the use of improper hindsight to combine the teachings of Köster et al. Gelfand et al. since neither of these references teaches or suggests (and at least one of the references actually teaches against) conversion of RNA to DNA "conducted in the presence of ...at least two thermostable DNA polymerases" as required by the present claims.

Therefore, as neither of Köster et al. nor Gelfand et al. teaches or suggests conversion of RNA to DNA "conducted in the presence of ...at least two thermostable DNA polymerases" and, similarly, as none of copending Application No. 09/257,166, Birch nor Hill teaches or suggests conversion of RNA to DNA "conducted in the presence of ...at least two thermostable DNA polymerases" as required by the present claims, Appellants respectfully submit that the presently claimed invention would not have been obvious over any combination of these references.

For all of the above noted reasons, it is strongly contended that certain clear differences exist between the present invention as claimed in claims 1-126, 132-137 and 141-146 and the prior art relied upon by the Examiner. It is further contended that these differences are more than sufficient that the present invention would not have been obvious to a person having ordinary skill in the art at the time the invention was made.

This final rejection being in error, therefore, it is respectfully requested that this honorable Board of Patent Appeals and Interferences reverse the Examiner's decision in this case and indicate the allowability of application claims 1-126, 132-137 and 141-146.

In the event that this paper is not being timely filed, the Appellants respectfully petition for an appropriate extension of time. Any fees for such an extension together

with any additional fees which may be due with respect to this paper may be charged to our Deposit Account No. 01-2300.

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APPENDIX

CLAIMS ON APPEAL

1. (Amended) A method for sequencing at least a portion of a RNA involving converting the RNA to a DNA and simultaneously amplifying the DNA and generating full length and truncated copies of the DNA for sequencing, comprising the steps of

(a) subjecting a mixture in a single step to a thermocycling reaction, the thermocycling reaction comprises heat denaturation, annealing and synthesis, wherein said mixture comprises

said RNA,

a buffer solution,

a first primer which is able to hybridize with a strand of said DNA,

a second primer which is able to hybridize with a strand of said DNA

complementary to the strand with which the first primer is able to hybridize, wherein at least one of the first and second primers is labeled,

deoxynucleotides or deoxynucleotide derivatives, wherein said deoxynucleotide

derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP,

at least one dideoxynucleotide or another terminating nucleotide, and at least two thermostable DNA polymerases, wherein said at least two

thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide compared with said first thermostable DNA polymerase, wherein one of said at least two thermostable DNA polymerases has reverse transcriptase activity,

to generate full-length and truncated copies of said DNA, wherein the full-length copies have a length equal to that of at least a portion of said DNA spanning the binding sites of the first and second primers;

(b) separating at least said truncated copies to make a sequence ladder; and thereafter

(c) reading the sequence ladder to obtain the sequence of said at least a portion of said RNA wherein the conversion of the RNA to the DNA is conducted in the presence of the at least two thermostable DNA polymerases.

2. (Original) The method of claim 1, wherein the deoxynucleotide derivatives are thionucleotides, 7-deaza-2'-dGTP, 7-deaza-2'-dATP or deoxyinosine triphosphate.

3. (Original) The method of claim 1, wherein said another terminating nucleotide is 3'-aminonucleotide or a nucleotide having an ester group at the 3' position.

4. (Original) The method of claim 1, wherein said first thermostable DNA polymerase has a reduced discrimination, compared with wild-type Taq DNA polymerase, against said dideoxynucleotide or another terminating nucleotide relative to deoxynucleotides or deoxynucleotide derivatives.

5. (Previously Presented) The method of claim 4, wherein said first thermostable DNA polymerase is a Taq DNA polymerase lacking 5'-3' exonuclease activity and having a Tabor-Richardson mutation.

6. (Previously Presented) The method of claim 5, wherein said first thermostable DNA polymerase is AmplitaqFS™, Taquenase™ or Thermo Sequenase™.

7. (Previously Presented) The method of claim 6, wherein said first thermostable DNA polymerase is Thermo Sequenase™.

8. (Original) The method of claim 1, wherein said second thermostable DNA polymerase has reverse transcriptase activity.

9. (Previously Presented) The method of claim 8, wherein said second thermostable DNA polymerase is Taq DNA polymerase, Tth DNA polymerase, Tfl DNA polymerase, or Klentaq (Taq DNA polymerase) (-exo5'-3'), or a DNA polymerase from Carboxydothermus hydrogenoformans having reverse transcriptase activity.

10. (Original) The method of claim 9, wherein said second thermostable DNA polymerase is Taq DNA polymerase, Tth DNA polymerase or Tfl DNA polymerase.

11. (Previously Presented) The method of claim 1, wherein said first thermostable DNA polymerase is Thermo Sequenase™, and said second thermostable DNA polymerase is Taq DNA polymerase.

12. (Previously Presented) The method of claim 11, wherein said second thermostable DNA polymerase is Tth DNA polymerase, and wherein step (a) is carried out in the presence of MnCl2 or Mn acetate.

13. (Original) The method of claim 1, wherein the thermocycling reaction in step (a) is carried out without interruption in a single container, vessel or tube.

14. (Original) The method of claim 1, wherein the ratio of said first primer to said second primer is not equal to 1:1.

15. (Original) The method of claim 14, wherein said ratio is between about 2:1 and about 3:1.

16. (Original) The method of claim 15, wherein said ratio is about 2:1.

17. (Original) The method of claim 1, wherein the first and second primers are differently labelled.

18. (Original) The method of claim 1, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least 55°C.

19. (Original) The method of claim 1, wherein said RNA in said mixture is a RNA of a single-copy gene.

20. (Original) The method of claim 1, wherein said mixture further comprises at least one thermostable pyrophosphatase.

21. (Original) The method of claim 1, wherein at least one of the first and second primers has a length that, in combination with a high annealing temperature, prevents annealing to unspecific DNA fragments during the heat denaturation of the thermocycling reaction.

22. (Original) The method of claim 21, wherein said length is at least 18 nucleotides.

23. (Original) The method of claim 1, wherein said RNA in said mixture is obtained from a body fluid, hairs, a cell, cells or fractions thereof, a tissue or fractions thereof, cell cultures or fractions thereof, bacteria or viruses.

24. (Original) The method of claim 1, wherein said RNA in said mixture is unpurified RNA.

25. (Original) The method of claim 24, wherein said RNA is total genomic RNA.

26. (Original) The method of claim 1, wherein said RNA in said mixture is a RNA of a single-copy gene and said mixture further comprises genomic DNA.

27. (Original) The method of claim 1, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least about 55°C.

28. (Original) The method of claim 27, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least about 68°C.

29. (Original) The method of claim 1, wherein the molar ratio of said deoxynucleotides or deoxynucleotide derivatives to said at least one dideoxynucleotide or another terminating nucleotide is between about 100:1 and about 1000:1.

30. (Original) The method of claim 29, wherein the molar ratio of said deoxynucleotides or deoxynucleotide derivatives to said at least one dideoxynucleotide or another terminating nucleotide is between about 300:1 and about 600:1.

31. (Original) The method of claim 1, wherein said deoxynucleotides or deoxynucleotide derivatives are present at a concentration of about 300mM to about 2 mM.

32. (Original) The method of claim 1, wherein said at least one dideoxynucleotide or another terminating nucleotide is present at a concentration of about 1mM to about 5mM.

33. (Original) The method of claim 1, wherein the length of said DNA is at least 500 nucleotides between the 3' ends of the first and second primers.

34. (Original) The method of claim 1, wherein said mixture further comprises at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule.

35. (Original) The method of claim 34, wherein said at least one polymerase-inhibiting agent inhibits at least said first thermostable DNA polymerase.

36. The method of claim 34, wherein said at least one polymerase-inhibiting agent inhibits at least said second thermostable DNA polymerase.

37. (Previously Presented) A kit for sequencing at least a portion of a RNA, comprising

deoxynucleotides or deoxynucleotide derivatives, which deoxynucleotide

derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP;

at least one dideoxynucleotide or another terminating nucleotide; and

at least two thermostable DNA polymerases, wherein said at least two

thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide in comparison to said first thermostable DNA polymerase, wherein at least one of said at least two thermostable DNA polymerases has reverse transcriptase activity;

wherein the at least two DNA polymers are mixed so that conversion of the RNA to the DNA will be conducted in the presence of the at least two thermostable DNA polymerases.

38. (Original) The kit of claim 37, wherein the deoxynucleotide derivatives are thionucleotides, 7-deaza-2'-dGTP, 7-deaza-2'-dATP or deoxyinosine triphosphate.

39. (Original) The kit of claim 37, wherein said another terminating nucleotide is 3'-aminonucleotide or a nucleotide having an ester group at the 3' position.

40. (Original) The kit of claim 37, wherein said first thermostable DNA polymerase has a reduced discrimination, compared with wild-type Taq DNA polymerase, against said dideoxynucleotide or another terminating nucleotide relative to deoxynucleotides or deoxynucleotide derivatives.

41. (Previously Presented) The kit of claim 40, wherein said first thermostable DNA polymerase is a Taq DNA polymerase lacking 5'-3' exonuclease activity and having a Tabor-Richardson mutation.

42. (Presently Presented) The kit of claim 41, wherein said first thermostable DNA polymerase is AmplitaqFS™, Taquenase™, or ThermoSequenase™.

43. (Previously Presented) The kit of claim 42, wherein said first thermostable DNA polymerase is ThermoSequenase™.

44. (Previously Presented) The kit of claim 37, wherein said second thermostable DNA polymerase is Taq DNA polymerase, Tth DNA polymerase, Tfl DNA polymerase, or Klentaq (Taq DNA polymerase) (-exo5'-3'), or a DNA polymerase from Carboxydothermus hydrogenoformans having reverse transcriptase activity.

45. (Previously Presented) The kit of claim 44, wherein said second thermostable DNA polymerase is Taq DNA polymerase.

46. (Original) The kit of claim 37, further comprising MnCl.sub.2 or Mn acetate, wherein said second thermostable DNA polymerase is Tth DNA polymerase.

47. (Original) The kit of claim 37, wherein the ratio of said first primer to said second primer is not equal to 1:1.

48. (Original) The kit of claim 47, wherein said ratio is between about 2:1 and about 3:1.

49. (Original) The kit of claim 48, wherein said ratio is 2:1.

50. (Original) The kit of claim 37, further comprising at least one thermostable pyrophosphatase.

51. (Original) The kit of claim 37, further comprises at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule.

52. (Original) The kit of claim 51, wherein said at least one polymerase-inhibiting agent inhibits at least said first thermostable DNA polymerase.

53. (Original) The kit of claim 51, wherein said at least one polymerase-inhibiting agent inhibits at least said second thermostable DNA polymerase.

54. (Original) The kit of claim 51, wherein said agent is an acid anhydride.

55. (Original) The kit of claim 54, wherein said agent is citraconic anhydride, cis-aconitic anhydride, phthalic anhydride, succinic anhydride or maleic anhydride.

56. The kit of claim 51, wherein said agent is a compound having at least one acid anhydride group per molecule.

57. (Original) The kit of claim 56, wherein said agent is a compound having two acid anhydride groups per molecule.

58. (Original) The kit of claim 57, wherein said agent is pyromellitic dianhydride or naphthalenetetracarboxylic dianhydride.

59. (Original) The method of claim 34, wherein said agent is an acid anhydride.

60. (Original) The method of claim 59, wherein said agent is citraconic anhydride, cis-aconitic anhydride, phthalic anhydride, succinic anhydride or maleic anhydride.

61. (Original) The method of claim 34, wherein said agent is a compound having at least one acid anhydride group per molecule.

62. (Original) The method of claim 61, wherein said agent is a compound having two acid anhydride groups per molecule.

63. (Original) The method of claim 62, wherein said agent is pyromellitic dianhydride or naphthalenetetracarboxylic dianhydride.

64. (Previously Presented) A method for sequencing at least a portion of a DNA involving simultaneously amplifying the DNA and generating full length and truncated copies of the DNA for sequencing, comprising the steps of (a) subjecting a mixture in a single step to a thermocycling reaction, the thermocycling reaction comprises heat denaturation, annealing and synthesis, wherein said mixture comprises said DNA, a buffer solution, a first primer which is able to hybridize with a strand of said DNA, a second primer which is able to hybridize with a strand of said DNA complementary to the strand with which the first primer is able to hybridize, wherein at least one of the first and second primers is labelled, deoxynucleotides or deoxynucleotide derivatives, wherein said deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP, at least one dideoxynucleotide or another terminating nucleotide,

at least two thermostable DNA polymerases, wherein said at least two thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide compared with said first thermostable DNA polymerase, and at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule, to generate full-length and truncated copies of said DNA, wherein the full-length copies have a length equal to that of at least a portion of said DNA spanning the binding sites of the first and second primers; (b) separating at least said truncated copies to make a sequence ladder; and thereafter (c) reading the sequence ladder to obtain the sequence of said at least a portion of said DNA.

65. (Original) The method of claim 64, wherein the deoxynucleotide derivatives are thionucleotides, 7-deaza-2'-dGTP, 7-deaza-2'-dATP or deoxyinosine triphosphate.

66. (Original) The method of claim 64, wherein said another terminating nucleotide is 3'-aminonucleotide or a nucleotide having an ester group at the 3' position.

67. (Original) The method of claim 64, wherein said first thermostable DNA polymerase has a reduced discrimination, compared with wild-type Taq DNA polymerase, against said dideoxynucleotide or another terminating nucleotide relative to deoxynucleotides or deoxynucleotide derivatives.

68. (Previously Presented) The method of claim 67, wherein said first thermostable DNA polymerase is a Taq DNA polymerase lacking 5'-3' exonuclease activity and having a Tabor-Richardson mutation.

69. (Previously Presented) The method of claim 68, wherein said first thermostable DNA polymerase is AmplitaqFS™, Taquenase™, Thermo Sequenase™.

70. (Previously Presented) The method of claim 69, wherein said first thermostable DNA polymerase is Thermo Sequenase™.

71. (Previously Presented) The method of claim 64, wherein said at least one polymerase inhibiting agent has at least one said anhydride group per molecule.

72. (Previously Presented) The method of claim 64, wherein said second thermostable DNA polymerase is Taq DNA polymerase, Tth DNA polymerase, Tfl DNA polymerase, or Klentaq (Taq DNA polymerase) (-exo5'-3').

73. (Original) The method of claim 72, wherein said second thermostable DNA polymerase is Taq DNA polymerase, Tth DNA polymerase or Tfl DNA polymerase.

74. (Previously Presented) The method of claim 64, wherein said first thermostable DNA polymerase is Thermo Sequenase™, and said second thermostable DNA polymerase is Taq DNA polymerase.

75. (Previously Presented) The method of claim 74, wherein said second thermostable DNA polymerase is Tth DNA polymerase and wherein step (a) is carried out in the presence of MnCl.$MnCl_2$ or Mn acetate.

76. (Original) The method of claim 64, wherein the thermocycling reaction in step (a) is carried out without interruption in a single container, vessel or tube.

77. (Original) The method of claim 64, wherein the ratio of said first primer to said second primer is not equal to 1:1.

78. (Original) The method of claim 77, wherein said ratio is between about 2:1 and about 3:1.

79. (Original) The method of claim 78, wherein said ratio is about 2:1.

80. (Original) The method of claim 64, wherein the first and second primers are differently labelled.

81. (Original) The method of claim 64, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least 55°C.

82. (Original) The method of claim 64, wherein said DNA in said mixture is a single-copy DNA in a complex mixture of DNA.

83. (Original) The method of claim 64, wherein said mixture further comprises at least one thermostable pyrophosphatase.

84. (Original) The method of claim 64, wherein at least one of the first and second primers has a length that, in combination with a high annealing temperature, prevents annealing to unspecific DNA fragments during the heat denaturation of the thermocycling reaction.

85. (Original) The method of claim 84, wherein said length is at least 18 nucleotides.

86. (Original) The method of claim 64, wherein said DNA in said mixture is obtained from a body fluid, hairs, a cell, cells or fractions thereof, a tissue or fractions thereof, cell cultures or fractions thereof, bacteria or viruses.

87. (Original) The method of claim 64, wherein said DNA in said mixture is unpurified DNA.

88. (Original) The method of claim 87, wherein said DNA is total genomic DNA.

89. (Original) The method of claim 64, wherein said DNA in said mixture is a single-copy DNA, wherein said mixture further comprises total genomic DNA.

90. (Original) The method of claim 64, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least about 55°C.

91. (Original) The method of claim 90, wherein said annealing and synthesis of the thermocycling reaction is carried out at a temperature of at least about 68°C.

92. (Original) The method of claim 64, wherein the molar ratio of said deoxynucleotides or deoxynucleotide derivatives to said at least one dideoxynucleotide or another terminating nucleotide is between about 100:1 and about 1000:1.

93. (Original) The method of claim 92, wherein the molar ratio of said deoxynucleotides or deoxynucleotide derivatives to said at least one dideoxynucleotide or another terminating nucleotide is between about 300:1 and about 600:1.

94. (Original) The method of claim 64, wherein said deoxynucleotides or deoxynucleotide derivatives are present at a concentration of about 300mM to 2 mM.

95. (Original) The method of claim 64, wherein said at least one dideoxynucleotide or another terminating nucleotide is present at a concentration of about 1 to 5mM.

96. (Original) The method of claim 64, wherein the length of the DNA in said mixture is at least 500 nucleotides between the 3' ends of the first and second primers.

97. (Original) The method of claim 64, wherein said at least one polymerase-inhibiting agent inhibits at least said first thermostable DNA polymerase.

98. (Original) The method of claim 64, wherein said at least one polymerase-inhibiting agent inhibits at least said second thermostable DNA polymerase.

99. (Previously Presented) The method of claim 64, wherein said at least one polymerase-inhibiting agent reversibly loses inhibitory activity at the temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule, thereby enabling said agent to inhibit said at least one of said at least two thermostable DNA polymerases in more than one thermocycle.

100. (Previously Presented) A kit for sequencing at least a portion of a DNA, comprising deoxynucleotides or deoxynucleotide derivatives, which deoxynucleotide derivatives are able to be incorporated by a thermostable DNA polymerase into growing DNA molecules in place of one of dATP, dGTP, dTTP or dCTP; at least one dideoxynucleotide or another terminating nucleotide; at least two thermostable DNA polymerases, wherein said at least two thermostable DNA polymerases are at least a first thermostable DNA polymerase and a second thermostable DNA polymerase, which second thermostable DNA polymerase has a reduced ability to incorporate said dideoxynucleotide or another terminating nucleotide in comparison to said first thermostable DNA polymerase; and at least one polymerase-inhibiting agent against at least one of said at least two thermostable DNA polymerases, wherein said at least one polymerase-inhibiting agent loses inhibitory ability, thereby allowing said at least one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule, wherein said at least one polymerase-inhibting agent is a compound having at least one acid anhydride group per molecule.

101. (Original) The kit of claim 100, wherein the deoxynucleotide derivatives are thionucleotides, 7-deaza-2'-dGTP, 7-deaza-2'-dATP or deoxyinosine triphosphate.

102. (Original) The kit of claim 100, wherein said another terminating nucleotide is 3'-aminonucleotide or a nucleotide having an ester group at the 3' position.

103. (Original) The kit of claim 100, wherein said first thermostable DNA polymerase has a reduced discrimination, compared with wild-type Taq DNA polymerase, against said dideoxynucleotide or another terminating nucleotide relative to deoxynucleotides or deoxynucleotide derivatives.

104. (Previously Presented) The kit of claim 103, wherein said first thermostable DNA polymerase is a Taq DNA polymerase lacking 5'-3' exonuclease activity and having a Tabor-Richardson mutation.

105. (Previously Presented) The kit of claim 104, wherein said first thermostable DNA polymerase is AmplitaqFS™, Taquenase™, or ThermoSequenase™.

106. (Previously Presented) The kit of claim 105, wherein said first thermostable DNA polymerase is ThermoSequenase™.

107. (Previously Presented) The kit of claim 100, wherein said second thermostable DNA polymerase is Taq DNA polymerase, Tth DNA polymerase, Tfl DNA polymerase, or Klentaq (Taq DNA polymerase) (-exo5'-3'),

or a DNA polymerase from Carboxydothermus hydrogenoformans having reverse transcriptase activity.

108. (Previously Presented) The kit of claim 107, wherein said second thermostable DNA polymerase is Taq DNA polymerase.

109. (Original) The kit of claim 100, further comprising MnCl.sub.2 or Mn acetate, wherein said second thermostable DNA polymerase is Tth DNA polymerase.

110. (Original) The kit of claim 100, wherein the ratio of said first primer to said second primer is not equal to 1:1.

111. (Original) The kit of claim 110, wherein said ratio is between about 2:1 and about 3:1.

112. (Original) The kit of claim 111, wherein said ratio is about 2:1.

113. (Original) The kit of claim 100, further comprising at least one thermostable pyrophosphatase.

114. (Original) The kit of claim 100, wherein said at least one polymerase-inhibiting agent inhibits at least said first thermostable DNA polymerase.

115. (Original) The kit of claim 100, wherein said at least one polymerase-inhibiting agent inhibits at least said second thermostable DNA polymerase.

116. (Original) The kit of claim 100, further comprises an antibody against one of said at least two thermostable DNA polymerases, wherein said antibody loses inhibitory ability, thereby allowing said one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule.

117. (Original) The kit of claim 116, wherein inhibition of said one of said at least two thermostable DNA polymerases by said antibody begins at a lower temperature than inhibition of said at least one of said at least two thermostable DNA polymerases by said polymerase-inhibiting agent.

118. (Original) The kit of claim 100, wherein said polymerase-inhibiting agent is citraconic anhydride, cis-aconitic anhydride, phthalic anhydride, succinic anhydride or maleic anhydride.

119. (Original) The kit of claim 100, wherein said polymerase-inhibiting agent is a compound having two acid anhydride groups per molecule.

120. (Original) The kit of claim 119, wherein said agent is pyromellitic dianhydride or naphthalenetetracarboxylic dianhydride.

121. (Previously Presented) The kit of claim 100, wherein said at least one polymerase-inhibiting agent reversibly loses inhibitory activity at the temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule, thereby enabling said agent to inhibit said at least one of said at least two thermostable DNA polymerases in more than one thermocycle.

122. (Original) The method of claim 64, wherein said mixture further comprises an antibody against one of said at least two thermostable DNA polymerases, wherein said antibody loses inhibitory ability, thereby allowing said one of said at least two thermostable DNA polymerases to be active, at a temperature which is at least the temperature at which unspecifically hybridized primers separate from a DNA molecule.

123. (Original) The method of claim 122, wherein inhibition of said one of said at least two thermostable DNA polymerases by said antibody begins

at a lower temperature than inhibition of said at least one of said at least two thermostable DNA polymerases by said polymerase-inhibiting agent.

124. (Previously Presented) The method of claim 71, wherein said polymerase-inhibiting agent is citraconic anhydride, cis-aconitic anhydride, phthalic anhydride, succinic anhydride or maleic anhydride.

125. (Previously Presented) The method of claim 71, wherein said agent is a compound having two acid anhydride groups per molecule.

126. (Original) The method of claim 125, wherein said agent is pyromellitic dianhydride or naphthalenetetracarboxylic dianhydride.

Claims 127-131 (Canceled)

132. (Previously Presented) The method of claim 64, wherein said mixture further comprises at least one agent that lowers the melting point of the DNA.

133. (Previously Presented) The method of claim 132, wherein said at least one agent is selected from the group consisting of glycerin, trehalose, betaine and DMSO.

134. (Previously Presented) The method of claim 97, wherein an inhibitory activity of said at least one polymerase-inhibiting agent is reversibly reduced at a specific temperature and after a specific number of thermocycles allowing sequencing of the DNA to start after the DNA has been amplified.

135. (Previously Presented) The method of claim 134, wherein the inhibitory activity of said at least one polymerase-inhibiting agent is reversibly reduced when the reaction mixture is exposed at an elevated temperature.

136. (Previously Presented) The method of claim 135, wherein said first thermostable DNA polymerase is a DNA polymerase which carries a Tabor-Richardson mutation and has no 5' to 3' exonuclease activity.

137. (Previously Presented) The method of claim 136, wherein said first thermostable DNA polymerase is selected from the group consisting of AMPLITAQ FS™, TAQUENASE™, and THERMOSEQUENASE.

Claims 138-140 (Canceled)

141. (Previously Presented) The kit of claim 100 further comprising at least one agent that lowers the melting point of the DNA.

142. (Previously Presented) The kit of claim 141, wherein said at least one agent is selected from the group consisting of glycerin, trehalose, betaine and DMSO.

143. (Previously Presented) The method of claim 8, wherein said mixture further comprises a polymerase-inhibiting agent against said second thermostable DNA polymerase.

144. (Previously Presented) The method of claim 143, wherein an inhibitory activity of said polymerase-inhibiting agent is reduced after reversed transcription of the RNA.

145. (Previously Presented) The kit of claim 124 wherein said polymerase-inhibiting agent is citraconic anhydride.

146. (Previously Presented) The method of claim 64 further comprising, prior to step (a), a step of mixing all of said DNA, said buffer solution, said first primer, said second primer, said deoxynucleotides or deoxynucleotide derivatives, said dideoxynucleotide or other terminating nucleotide, the at least two thermostable DNA polymerases and the at least one polymerase-inhibiting agent in a single container, vessel or tube.